



Physicochemical Properties and End-use Quality of Wheat Starch as a Function of Waxy Protein Alleles

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ABSTRACT

Soft wheat (*Triticum aestivum* L.) quality tests (milling and baking quality) and starch characteristics (amylose concentration, X-ray diffractograms, thermal properties and pasting properties) were determined for eight granule-bound starch synthase (GBSS; waxy protein) genotypes in a soft wheat background. Lines carrying two null alleles showed reduced amylose concentrations relative to those of single null and wild-type lines. Milling and baking quality traits were clearly different between waxy (triple null) and the other genotypes. Waxy lines showed the highest alkaline water retention (AWRC) capacity; even though, the protein concentration was not significantly different from some double null and single null lines. The typical A-type patterns of X-ray diffractograms were observed for all starches. Waxy starch showed higher crystallinity than non-waxy starches. Analysis by Rapid Viscoanalyser (RVA) showed distinctive differences among the eight genotypes. Waxy starches showed higher peak viscosity, lower peak temperature and shorter peak time than those of all other genotypes. The results suggest that baking quality of waxy wheat can not be predicted by either AWRC or protein concentration. The interactions based on biochemical analysis between protein and other fractions (amylose and amylopectin, damaged starch) of partially and fully waxy wheat flours must be assessed before baking quality can be predicted. Furthermore, waxy wheat may not be suitable for current application of soft wheat products; however, distinct properties of waxy starch may open the possibility of commercial use in novel applications. Also, double null and single null genotypes may be a good source of variation for specific end-use products.

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INTRODUCTION

Starch is the primary component of wheat endosperm (*Triticum aestivum* L.) and the characteristics of the starch is an important factor controlling the texture of noodles^{1,2} and other products. In wheat plants, starch is synthesized in amyloplasts. Within the amyloplasts, granule-bound starch synthase (GBSS; EC 2.4.1.21) is the primary enzyme responsible for amylose synthesis. Amylopectin syn-

thesis involves starch synthases, branching and de-branching enzymes³. The genetic loci (*wx*) encoding isoforms of GBSS are localized on chromosomes 7AS (*wx-A1*), 4AL (*wx-B1*) and 7DS (*wx-D1*) in hexaploid wheat⁴. The locus of *wx-B1* was originally located on chromosome 7BS and a reciprocal translocation occurred between chromosomes 7B and 4A during evolution. Wheat with the three GBSS null alleles produces fully waxy starch with no or little amylose. Mutants (null alleles) resulting in the loss of one or two GBSS isoforms have been termed 'partially waxy'. Waxy wheat has been created using hybridization, mutagenesis, and somaclonal

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mutation⁵⁻⁷. The partially waxy condition results in reduced amylose concentration in starch.

The physicochemical properties of waxy wheat have not been defined clearly, although it has been reported that the end-use quality of waxy wheat may not be suitable for present applications such as cookie and bread baking, and noodle making⁸. Different tests, such as X-ray diffractograms, differential scanning calorimetry (DSC), Brabender Viscoanalyser (BVA) and the Rapid Viscoanalyser (RVA), have been applied to study the physicochemical properties of waxy starch^{7,9,10}. The crystalline ratio, thermal transition temperatures, and viscosity of starch showed different patterns between waxy and normal starch. However, no differences were observed for amylopectin branching frequencies⁸ and the degree of polymerization of amylopectin chains among waxy, partially waxy, and wild-type starches.

In this study, eight genotypes were produced by crossing Kanto107, Bai-Huo and an Arkansas soft wheat line. End-use quality of flour and physicochemical properties of starch were analyzed. The goal of this study was to understand the influence of *wx* genes on the physicochemical properties of starch and end-use quality in a soft red wheat genetic background.

MATERIALS AND METHODS

Wheat samples

The cross: Kanto107/Bai-Huo/3/Bai-Huo/Kanto107//A92-3327/Kanto107, was used to develop segregating populations. Kanto107 is a Japanese *wx-A1/wx-B1* double null white wheat cultivar. Bai-Huo is Chinese *wx-D1* null white wheat. A92-3327 is wild-type Arkansas soft wheat. Seventy F3 lines were developed in the greenhouse. Four seeds per F3 line were bulked and tested for GBSS isoforms. Three seeds per F3 line were randomly selected and advanced to the F4 generation in the greenhouse and planted as head rows at Plains, Georgia in 1999. One hundred and fifty F5 lines were harvested, and eight seeds per F5 line were individually tested for GBSS isoforms to discard heterozygous lines. Forty-four homozygous F5 lines were selected and grouped as eight genotypes based on the absence of one or more isoforms of GBSS: fully waxy (*wx-A1/wx-B1/wx-D1* null), *wx-B1/wx-D1* null, *wx-A1/wx-D1* null, *wx-A1/wx-B1* null, *wx-D1* null, *wx-B1* null, *wx-A1* null, and wild-type. Two lines per genotype were randomly selected and

processed to extract starch. Thirty lines were tested for milling and baking quality and starch properties.

Quality tests

Five *wx-B1/wx-D1* null lines, three *wx-A1/wx-D1* null lines, five *wx-A1/wx-B1* null lines, two *wx-D1* null lines, three *wx-B1* null lines, three *wx-A1* null lines, three wild-type lines, and six waxy lines were tested for soft wheat quality traits. Grain samples (100 g) were analyzed at the USDA-ARS Soft Wheat Quality Lab., Wooster, OH, USA, for milling (adjusted flour yield and softness equivalent) and baking (flour protein, alkaline water retention capacity and lactic acid retention capacity) quality. Shriveled seeds were removed from each sample before analyses. Approved Method 26-32 was used for evaluating adjusted flour yield¹¹. Softness equivalent (SE) was measured according to Gaines *et al.*¹². Approved Methods 46-10, 54-50 and 56-11 were used to determine protein concentration, AWRC (alkaline water retention capacity) and LARC (lactic acid retention capacity)¹¹.

GBSS identification

GBSS isoforms were identified from both single seed and bulk samples. For single seed analysis, seeds (brush end) were crushed and placed in a 1.5 microcentrifuge tube with 1 ml Tris-HCl (0.6 M), pH 6.8 and 2 µl ficin to each tube. The tube was vortexed and incubated at 37 °C overnight. The supernatant was discarded after centrifugation at 12 000 × *g* for 10 s. After adding 1 ml GBSS extraction buffer (0.055 M Tris-HCl, pH 6.8, 2.6% SDS, 10% glycerol, 2% β-mercaptoethanol), the tube was completely vortexed and incubated in a 37 °C orbital shaker for 2 h. The supernatant was discarded after centrifugation at 12 000 × *g* for 10 s. After washing with 1 ml of double distilled water twice, repeated pellet washes were conducted with two changes of acetone. After washing and centrifugation, the pellet was placed in a hood to dry.

To identify segregating genotypes, 10 mg of starch from single seed extractions was transferred into a 0.5 ml micro-tube. Starch was completely gelatinized by boiling for 10–15 min with 100 µl of Lane buffer (2% SDS, 10% glycerol, 0.06 M Tris-HCl, pH 8.8, 0.002 M Ethylenediaminetetraacetic acid) and 10 µl dithioerythritol (from stock solution of 60 mg/ml). After boiling, 200 µl of 4-vinylpyridine (4-vp) solution (2.2 µl 4-vp/1 ml lane buffer) was added and mixed completely by vortexing. The

sample tube was placed on a 55 °C heating block for 30–45 min. An additional 10 µl of Bromophenol Blue solution was added, and the tube was vortexed and placed on a 55 °C heating block for at least 15 min. Before loading into gels, samples were centrifuged at $14\,000 \times g$ for 3 min. The GBSS isolated from starch was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver-stained, using an acrylamide-bisacrylamide concentration of 30:1¹³. Samples were electrophoresed in a 17% gel at 20 watts for 5 h.

Bulk isolation of starch

Starch was extracted with GBSS extraction buffer from ground samples to examine the physicochemical properties of starch. Five grams of whole wheat kernels were ground slightly in a coffee grinder before steeping in 50 ml of extraction buffer for 24 h and shaking. Liquid was decanted followed by centrifugation at $3000 \times g$ for 5 min. Another extraction was done by adding 50 ml of extraction buffer and shaking. After centrifugation, the pellet was washed two times by adding 50 ml of water and shaking. The slurry was filtered through a cheese cloth with additional water until no more starch was released. After centrifugation, the pellet was washed with two changes of acetone. The pellet was then air dried.

Amylose content and α -amylase activity

Amylose content of starch was determined by the method of Knutson and Grove¹⁴. α -Amylase activity in flour was determined by the method of McCleary and Sheehan¹⁵ using the Megazyme (Bray, Ireland) kit.

Scanning electron microscopy

Scanning electron micrographs (SEM) of the starches were taken with a scanning electron microscope (LEO 982, Field Emission SEM) located at the Ultrastructural Research Center, University of Georgia. Starch samples were coated with gold. Micrographs of each sample were taken at $1500\times$ magnification.

X-ray diffraction

X-ray diffraction patterns of starch samples (1.5 g) were investigated with Co K α radiation using a diffractometer (Scintag XDS 2000), operated at 40 kV and 40 mA. The scanning region of the two-theta angle (2θ) was from 10 to 40 degrees

with a 0.02 step size and a count time of 1°/min. Diffraction optic included 250 mm focusing circle, 1°/2° primary/scatter slits, and 0.5°/0.3° scatter/receiving slits. The relative crystallinity (degree of crystallization) of starch was estimated¹⁶.

Thermal properties and pasting properties

Thermal properties of starches were analyzed by a differential scanning calorimeter (DSC-7, Perkin Elmer, Norwalk, CT, USA) equipped with an intracooling II system. Indium and distilled water were used as the reference standards. Approximately 4 mg of starch and 12 mg of distilled water were weighed into aluminum pans (Perkin Elmer) and sealed. The heating rate was at 10 °C/min over the temperature range of 0–80 °C. Pasting properties of starch were measured with a Rapid Viscoanalyzer (RVA, Newport Scientific, Sydney, Australia). 2.5 g of starch were dispersed in 25 ml of distilled water. The rotating speed of the paddle was 160 rev/min except for the first 10 s (960 rev/min). The suspension was equilibrated at 50 °C for 1 min and heated at a rate of 12 °C/min to 95 °C and then held at 95 °C for 2.5 min. The sample was then cooled to 50 °C at a rate of 12 °C/min and then held for 3 min at 50 °C. The data presented were averages of three replicates for each starch sample. Pasting temperature, peak viscosity, peak temperature, peak time, minimum viscosity, break down, final viscosity and set back were obtained.

RESULTS

Amylose content and α -amylase activity

The average amylose content of waxy starch (Table I) was 0.9%. The amylose concentration of double null genotypes was significantly lower than that of single null and wild-type genotypes. Because the LSD value of the amylose determinations was high, no significant differences were observed amongst the three single null genotypes, or within the double null genotypes. The α -amylase activities of flour samples ranged from 0.16 to 0.78 CU (Ceralpha units) compared to 340 CU for Megazyme's malt standard. No samples showed high amylase activity.

Milling and baking quality

Adjusted flour yield, SE (Softness Equivalent), flour protein, AWRC and LARC are presented in Table I. Waxy lines showed the lowest flour yield of 69.5%.

Table I Milling and baking quality traits of waxy gene segregants

Genotype	%					
	Starch amylose	Adjusted flour yield	SE ^a	Flour protein	AWRC ^b	LARC ^c
Waxy (6) ^d	0.9 ^d	69.5 ^d	39.2 ^c	12.1 ^a	71.3 ^a	97.7 ^a
<i>wx-B1/D1</i> null (5)	19.6 ^c	71.7 ^{a-c}	41.2 ^{bc}	11.7 ^{ab}	62.5 ^b	94.0 ^{ab}
<i>wx-A1/D1</i> null (3)	21.1 ^c	71.8 ^{a-c}	40.9 ^{bc}	11.4 ^{bc}	60.6 ^{b-d}	85.9 ^{bc}
<i>wx-A1/B1</i> null (5)	20.1 ^c	72.0 ^{a-c}	40.7 ^{bc}	11.7 ^{ab}	61.5 ^{bc}	81.1 ^c
<i>wx-D1</i> null (2)	24.5 ^{ab}	73.0 ^{ab}	44.5 ^a	11.1 ^c	59.2 ^{de}	92.0 ^{a-c}
<i>wx-B1</i> null (3)	23.7 ^b	71.6 ^{bc}	41.9 ^b	12.1 ^a	61.4 ^{b-d}	98.2 ^a
<i>wx-A1</i> null (3)	24.1 ^{ab}	71.2 ^c	44.8 ^a	11.4 ^{bc}	60.1 ^{cd}	98.1 ^a
wild-type (3)	25.8 ^a	73.2 ^a	45.7 ^a	10.9 ^c	57.4 ^e	83.5 ^{bc}
LSD	2.1	1.5	2.6	0.6	2.3	11.3

^a Softness equivalent.^b Alkaline water retention capacity.^c Lactic acid retention capacity.^d Number of lines tested for milling and baking quality traits.^e Values with the same letter in the same column do not differ significantly at $P < 0.05$.

Wild-type, *wx-D1* null, and *wx-A1* null genotypes had significantly higher SE values. Waxy lines had harder grain texture (e.g. lower SE values) compared to wild-type and single null genotypes. Flour protein concentration was slightly higher in the waxy and *wx-B1* null genotypes compared to wild-type. AWRC, which typically is considered an indirect measure of protein quality, was higher in the waxy genotype, as compared to the other genotypes. Hence, amylose content must also contribute to variation in AWRC. The *wx-A1/wx-B1* double null showed lower LARC, while no significant differences were observed amongst waxy, *wx-B1* null and *wx-A1* null genotypes.

Granule morphology and crystalline structure of starch

Wheat starch granules showed bimodal size distributions (Fig. 1). A-granules were 17–32 μm , and B-granules were 2–8 μm in diameter. In all genotypes, the number of B-granules appeared to exceed the number of A-granules. No obvious morphological differences were observed in the starch granules of the waxy or partially waxy wheats.

The typical A-type patterns of X-ray diffractograms were observed (Fig. 2). The calculated crystallinities (degree of crystallization) of the samples are shown in Table II. Waxy starch showed no intensity peak at $2\theta = 23^\circ$ which represented the presence of the amylose-lipid complex¹⁷. Waxy starch showed higher crystallinity (34.8%) than non-waxy starches (Table II). Compared to other non-waxy genotypes

(20.4–23.4%), *wx-B1/wx-D1* double null also showed relatively higher crystallinity (28.3%).

Pasting properties and thermal transition

RVA of the starches showed distinctive differences in their pasting properties (Table II). The pasting temperature was lower for waxy starch. However, the range of pasting temperatures among all eight genotypes was less than 2°C . The average peak viscosities were 302 RVU for waxy starches and ranged from 197 to 216 for non-waxy starches. The *wx-B1/D1* double null and the *wx-A1/B1* double null had significantly different peak viscosities compared to the *wx-A1/D1* double null. Also the *wx-B1* single null had significantly higher peak viscosity than the other single null types (*wx-A1* null and *wx-D1* null). Peak temperature was lower for waxy starches. The average peak time was 2.8 min for waxy starches and 3.5–3.7 min for non-waxy starches. Waxy lines had higher minimum viscosity levels than the remaining genotypes. The average breakdown viscosities were 219 RVU for waxy starches and 153–171 RVU for non-waxy starches. The average final viscosities were lower for the *wx-A1/D1* double null and higher for wild-type starches. The average setback viscosities were 28 RVU for waxy starches and 56–67 RVU for non-waxy starches. There was a trend of higher peak viscosity, breakdown, final viscosity, and setback for *wx-B1/D1* and *wx-A1/B1* double null lines. The *wx-B1* null had higher peak viscosity and breakdown, and lower pasting temperature, final viscosity, and setback than the other two single null lines.

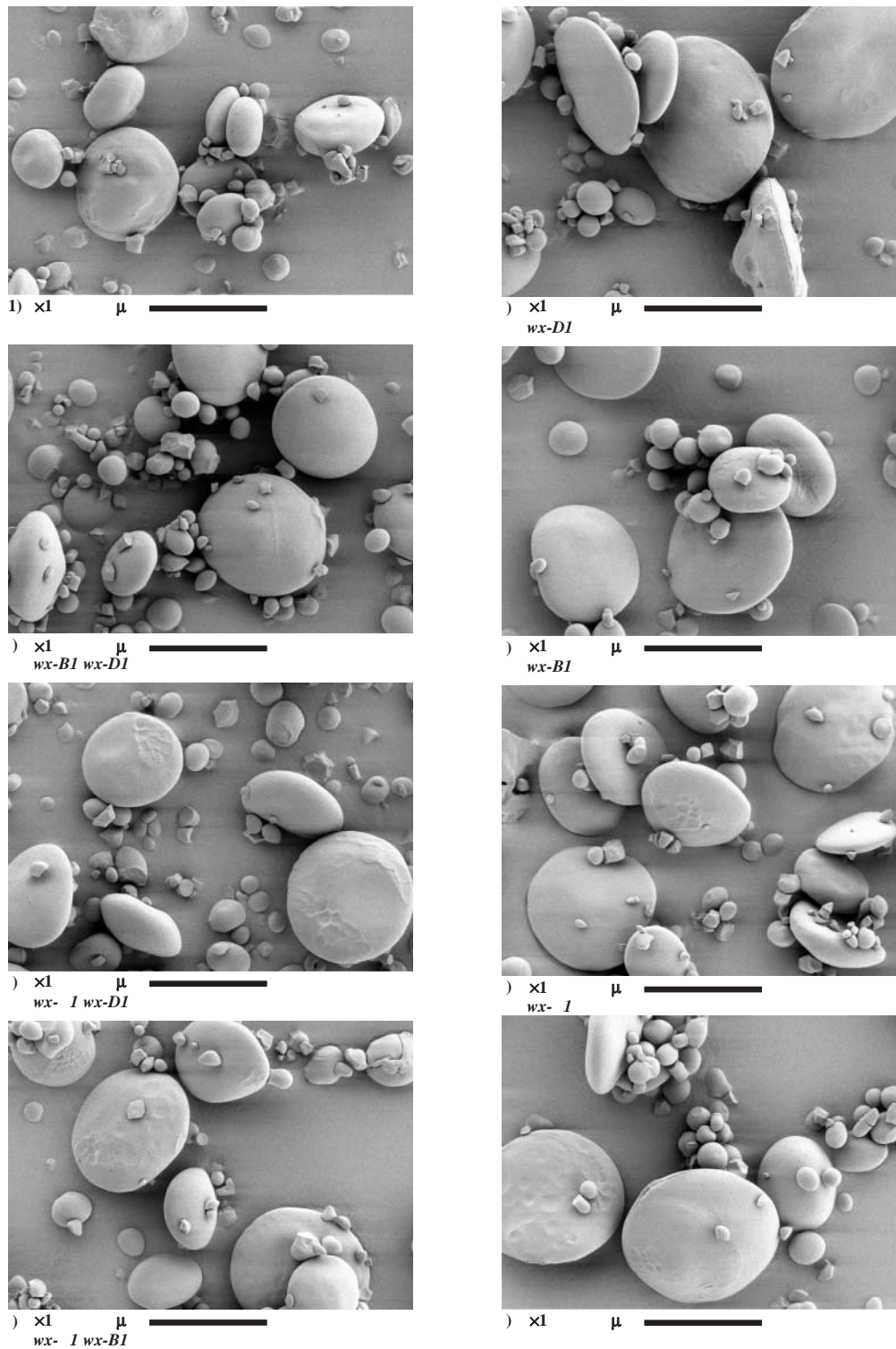


Figure 1 Scanning electron micrographs of wheat starches.

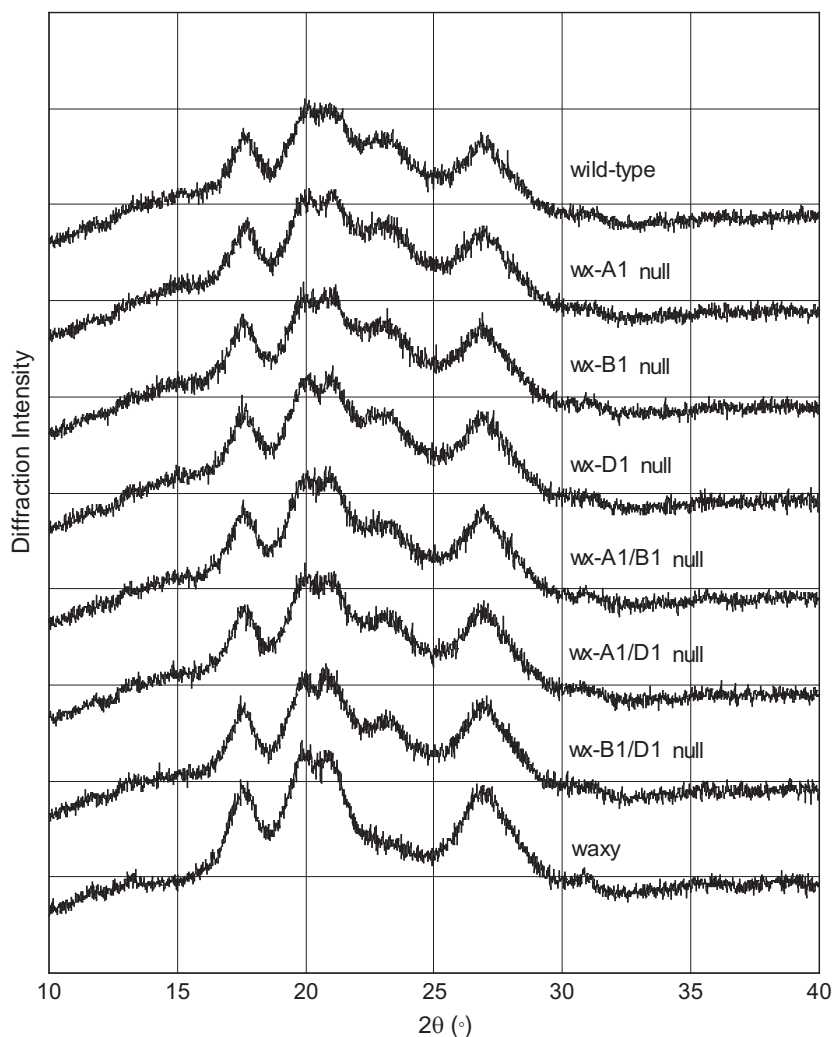


Figure 2 X-ray patterns of wheat starches.

Transition temperatures and enthalpies from the DSC study are summarized in Table III. Waxy starch had lower onset and peak temperature than those of non-waxy starches. Starches from *wx-B1/D1* and *wx-A1/B1* double null lines had higher enthalpy than those from other genotypes. No other differences were detected.

Correlations of starch physicochemical and functional properties

Simple correlations were used to examine relationships between physicochemical attributes and starch functional properties. Since the physicochemical and functional properties of waxy starch were quite different from amylose-containing starch, waxy

starches can be considered as outliers. Therefore, the non-waxy (amylose-bearing) group was examined separately. Separate correlations were calculated for the two groups 'non-waxy' (double null + single null + wild-type) and 'all-genotypes' (waxy + double null + single null + wild-type). Amylose concentration was correlated with crystallinity and starch pasting properties (Table IV). Negative correlations were observed in the non-waxy and all-genotypes groups. No significant relationship was observed for pasting temperature in the non-waxy group. Peak viscosity showed a negative correlation with amylose concentration in both groups. Positive correlations were observed for peak temperature, peak time and setback viscosity in both groups. No significant correlation was observed for minimum viscosity in

Table II Crystalline and starch-pasting properties (RVA) of waxy loci genotypes

Genotype	Crystalline (%)	Pasting temp (°C)	Peak viscosity (RVU)	Peak temp (°C)	Peak time (Minute)	Minimum viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)
Waxy	34.8	65.2c ^a	302a	71.8d	2.8d	83.1a	219a	110a–c	26.7e
<i>wx-B1/D1</i> null	28.3	67.2a	215b	80.0c	3.5c	47.7b	167b	107bc	59.7c
<i>wx-A1/D1</i> null	23.2	66.3ab	198c	80.7bc	3.5bc	45.0bc	153c	101d	55.5d
<i>wx-A1/B1</i> null	23.4	66.3ab	216b	80.1c	3.5c	45.1bc	171b	106bc	60.6bc
<i>wx-D1</i> null	20.4	66.7ab	197c	82.5a	3.7a	43.4c	154c	108c	64.2ab
<i>wx-B1</i> null	20.5	65.9bc	211b	80.6bc	3.5bc	44.2bc	166b	105cd	61.3bc
<i>wx-A1</i> null	22.5	66.3ab	200c	81.7ab	3.6ab	43.9bc	156c	111ab	66.6a
wild-type	21.6	66.5ab	201c	82.8a	3.7a	47.6b	153c	113a	65.5a
LSD		0.9	8.1	1.6	0.1	3.9	8.8	4.9	3.7

^a Values with the same letter in the same column do not differ significantly at $P < 0.05$.

Table III Thermal transition temperatures (°C)^a and enthalpies^b of waxy loci genotypes

Genotype	T_o	T_p	ΔH
Waxy	52.3c ^c	57.6e	9.2d
<i>wx-B1/D1</i> null	55.4a	60.1a	11.0a
<i>wx-A1/D1</i> null	54.3ab	59.4b	10.9ab
<i>wx-A1/B1</i> null	54.5ab	59.2b	11.1a
<i>wx-D1</i> null	54.3b	58.7c	10.2bc
<i>wx-B1</i> null	54.2b	59.3b	9.6cd
<i>wx-B1</i> null	53.6b	58.0d	9.8cd
wild-type	53.6b	57.9d	9.8cd
LSD	1.2	0.2	0.8

^a T_o = onset, T_p = peak.

^b J/g of starch.

^c Values with the same letter in the same column do not differ significantly at $P < 0.05$.

the non-waxy group. Breakdown viscosity showed significant and negative correlations with amylose concentration in both groups. Final viscosity showed a positive correlation with amylose concentration in the non-waxy group only.

Amylose concentration showed positive relationships with SE in the non-waxy and all-genotypes groups (Table V). A negative correlation was observed between amylose concentration and protein concentration in the all-genotypes group only. AWRC and crystallinity showed significant negative correlations with amylose concentration in both the non-waxy and all-genotypes groups. Flour yield showed a non significant relationship with all milling and baking quality traits in the non-waxy group.

SE showed a significant relationship with flour protein and AWRC in both groups. SE also showed a significant relationship with crystallinity in the all-genotypes group, while no significant correlation

was observed in the non-waxy group. Flour protein was highly correlated with AWRC in both groups. AWRC was significantly correlated with LARC in the all-genotypes group. Crystallinity showed significant correlation with AWRC in the non-waxy and in the all-genotypes groups, but this relationship may be caused by a stronger relationship between amylose concentration and crystallinity. LARC showed a significant relationship with baking quality score in the all-genotypes groups, while no significant correlation was detected in the other group. Baking quality score and crystallinity had a significant and negative correlation in both groups.

DISCUSSION

Amylose levels in the wheat genotypes examined were dependent, in part, on the number of active alleles producing GBSS isoforms. The starch of double null lines showed lower amylose concentration than single null lines and wild-type lines. This result agreed with the results of Graybosch *et al.*¹⁸ and Yamamori *et al.*⁴ However, the LSD of the amylose test was somewhat high; hence, the true effect of the GBSS alleles could not be clearly established.

Miura and Sugawara¹⁹ found that removal of chromosome 4A (*wx-B1* locus) reduced amylose concentration ~3%, while no or little reduction of amylose concentration was observed after removing either *wx-A1* or *wx-D1* through chromosomal manipulations Yamamori and Quynh²⁰ also reported that the *wx-B1* null induced lower amylose concentrations than did the *wx-D1* null and the *wx-A1* null. They also analyzed the effects of GBSS isomers based on amylose concentration and ranked the single null genotypes as *wx-B1* null < *wx-D1*

Table IV Simple correlations between amylose content, granule crystallinity and starch pasting properties as measured by RVA

Genotype	Crystallinity	Pasting temp	Peak viscosity	Peak time	Minimum viscosity	Break down	Final viscosity	Set back
Non-waxy ^a ₍₁₄₎ ^b	-0.77**	-0.13	-0.58*	0.79**	-0.28	-0.56*	-0.42	0.67*
All-genotypes ₍₁₇₎	-0.94**	0.53*	-0.96**	-0.96**	-0.97**	-0.93**	-0.13	0.97**

^a Non-waxy = double null + single null + wild-type.^b Number of lines tested.**Table V** Simple correlations for amylose content, milling and baking quality traits and crystallinity of starch of waxy loci genotypes

	Genotype	Flour yield	SE ^a	Flour protein	AWRC ^b	LARC ^c	Crystallinity
Amylose	Non-waxy ^d ₍₁₄₎ ^e	0.27	0.72**	-0.35	-0.67**	0.16	-0.77**
	All-genotypes ₍₁₇₎	-0.26	0.63**	-0.48**	-0.95**	-0.32	-0.94**
Flour Yield	Non-waxy ₍₂₄₎		0.13	-0.18	-0.32	-0.39	0.17
	All-genotypes ₍₃₀₎		0.46**	0.41*	-0.75**	-0.46*	-0.70**
SE	Non-waxy ₍₂₄₎			-0.68**	-0.83**	0.01	-0.38
	All-genotypes ₍₃₀₎			-0.73**	-0.74**	-0.12	-0.60**
Flour Protein	Non-waxy ₍₂₄₎				0.74**	0.23	0.17
	All-genotypes ₍₃₀₎				0.65**	0.34	0.44*
AWRC	Non-waxy ₍₂₄₎					0.34	0.52**
	All-genotypes ₍₃₀₎					0.43*	0.90**
LARC	Non-waxy ₍₂₄₎						0.05
	All-genotypes ₍₃₀₎						0.34

*** Significantly different at the 0.05 and 0.01 levels of probability, respectively.

^a Softness equivalent.^b Alkaline water retention capacity.^c Lactic acid retention capacity.^d Non-waxy = double null + single null + wild-type.^e Number of lines tested.

null < *wx-A1* null. This result was not confirmed in the present study, as the *wx-D1* null was found to have a higher amylose concentration than the *wx-A1* null. However, inconsistent evidence of the effects of the missing *wx-B1* locus also has been published. Zhao *et al.*²¹ reported that some *wx-B1* null lines induced higher amylose concentration than some wild-type lines, although they also observed a reduction of amylose concentration in many *wx-B1* null lines. Amylose content no doubt is influenced, as are most grain quality traits, both by major genes, genetic background, and cultural environments. Hence, inconsistent results with single null lines are not unexpected. In most studies, the presence of double nulls is more likely to result in significant reductions of amylose concentrations.

In the present study, waxy lines had a lower SE than wild-type and partial null genotypes. The flour yield of waxy lines also was lower than other lines, and the SE test showed no significant relationship with flour yield. Graybosch *et al.*²² suggested that

flour yield may not be a proper indicator of grain hardness. The waxy character and grain hardness are, however, independent of each other. Both hard and soft textured waxy wheat germplasm lines have been released²³. Bettge *et al.*²⁴ reported a differential response of starch granules to mechanical damage between waxy and normal wheat. Waxy wheat starch showed a higher susceptibility to mechanical damage, and, consequentially, greater damaged starch than normal wheat starch. This property might have contributed to the lower SE of waxy wheat. The measurement of SE is based on milling properties¹², and might not be indicative of grain hardness in this case.

Gluten proteins play an important role in wheat flour processing as they are required to form cohesive, elastic, and extensible dough. A high concentration of strong gluten produces noodles with an elastic texture²⁵, which is considered as poor quality for leavened soft wheat products. Lines with higher protein concentration showed higher AWRC.

The possible unsuitability of waxy wheat for current wheat end-products indicates protein alone does not determine flour quality. Park⁸ reported that waxy wheat induced lower bread volume, poor crumb firmness, smaller cookie diameter, poor top grain score and hard snapping force. Poor bread baking quality of waxy wheat may be caused by lack of amylose concentration and excessive damaged starch rather than protein concentration or protein quality. Therefore, baking quality of waxy wheat can not be predicted by either AWRC or protein concentration. The interactions between protein and other fractions (amylose and amylopectin, damaged starch) of partial and fully waxy wheat flours should be determined to predict baking quality.

The higher crystallinity of waxy starch reflects not only a higher amylopectin concentration but also a closely-packed crystal structure²⁶ in the starch granules. Waxy starch showed the highest crystallinity when compared to non-waxy starches. This result is consistent with the reports of Fujita *et al.*²⁶ and Hayakawa *et al.*⁹ who found that waxy starch was more crystalline than non-waxy starch due to lack of amylose in the starch. Increasing the amylose concentration in partially waxy wheat may decrease the crystallinity. The result of X-ray diffraction analysis of barley starches also showed higher crystallinity of waxy starch compared to normal and high amylose starch²⁷.

RVA tests of waxy starch showed distinct differences between normal and waxy starches. Pasting temperatures of waxy starches were lower than non-waxy starches in agreement with the results of Hayakawa *et al.*⁹ and Park⁸. The peak viscosities of waxy starches were higher than amylose-containing starches, in agreement with the reports by Reddy and Sieb²⁸ and Park⁸. Song and Jane²⁷ also reported higher peak viscosity values for waxy barley compared to normal and high amylose cultivars. Tester and Morrison²⁹ suggested that amylopectin is largely responsible for granule swelling. Waxy starch may take up water more quickly than normal starch and induce higher swelling in a shorter time and lower temperature. Hayakawa *et al.*⁹ reported contrasting results for the peak viscosity of waxy starch compared to normal starches.

Lines with *wx-B1* null had higher peak viscosity and lower peak temperature than wild-type lines. Zhao *et al.*³⁰ reported that starches of *wx-B1* null lines with lower average amylose concentration had higher peak viscosity and flour swelling volume compared to those of wild-type. Even though *wx-B1* nulls had higher amylose concentration than double nulls,

their peak viscosity, peak temperature and peak time were similar. This may be caused by a differential effect of each waxy allele, with the *wx-B1* null having a greater effect on both amylose content and functional properties than nulls at the other two loci.

Thermal transition studies indicated that onset (T_o), peak temperatures (T_p) and enthalpy (ΔH) of waxy starch were significantly lower than that of double null lines. Starch extracted from double nulls showed higher T_o , T_p and ΔH than did wild-type. This result is consistent with those of Demeke *et al.*³¹. The pasting temperature of starches showed a consistent trend with the results of DSC. In both tests, waxy starch initiated swelling at lower temperatures than the other genotypes.

CONCLUSION

Significant differences were detected in amylose contents and functional properties amongst the eight possible genotypes at the wheat *waxy* loci. In general, as the number of null alleles increased, amylose content decreased and starch crystallinity increased. However, the null allele at the *wx-B1* locus was found to have a greater effect on starch amylose content than single null alleles at the remaining two loci. No obvious differences were detected in the physical appearance of the starch granules of waxy *vs.* non-waxy starches, but waxy starches differed markedly in physicochemical and functional properties. The impact of waxy null alleles on such properties demonstrates that wheat quality no longer can be considered to be largely determined by protein quantity and quality. Interactions between protein and other fractions (amylose and amylopectin, damaged starch) of partially and fully waxy wheat flours must be considered before attempts are made to predict baking quality. The unique properties of waxy starch might render it unsuitable for applications in current soft wheat products, or in such products produced by traditional methods. Nonetheless, both waxy and partially waxy starches offer unique functional properties that might extend the use of wheat starches in both food and industrial products.

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